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(54) Title: HIV ENVELOPE V3-CCR5 BINDING SITE IMMUNOGEN

(57) Abstract: The present invention relates, in general, to an immunogen and, in particular, to an immunogen for inducing antibodies that neutralize a wide spectrum of HIV primary isolates. The invention also relates to a method of inducing anti-HIV antibodies using same.

HIV ENVELOPE V3-CCR5 BINDING SITE IMMUNOGEN

This application claims priority from US Provisional Application No. 60/333,148, filed November 27, 2001, the entire content of which is incorporated herein by reference.

TECHNICAL FIELD

The present invention relates, in general, to an immunogen and, in particular, to an immunogen for inducing antibodies that neutralize a wide spectrum of HIV primary isolates. The invention also relates to a method of inducing anti-HIV antibodies using such an immunogen.

BACKGROUND

As the HIV epidemic continues to spread world-wide, the need for an effective HIV vaccine remains urgent. A key obstacle to HIV vaccine development is the extraordinary variability of HIV and the rapidity and extent of HIV mutation (Wain-Hobson in The Evolutionary biology of Retroviruses, SSB Morse Ed. Raven Press, NY, pgs 185-209 (1994)).

Myers, Korber and colleagues have analyzed HIV sequences worldwide and divided HIV isolates into groups or clades, and provided a basis for evaluating the evolutionary relationship of individual HIV isolates to each other (Myers et al (Eds), Human Retroviruses and AIDS (1995), Published by Theoretical Biology and Biophysics Group, T-10,

Mail Stop K710, Los Alamos National Laboratory, Los Alamos, NM 87545). The degree of variation in HIV protein regions that contain CTL and T helper epitopes has also recently been analyzed by Korber et al, and sequence variation documented in many CTL and T helper epitopes among HIV isolates (Korber et al (Eds), HIV Molecular Immunology Database (1995), Published by Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545).

A new level of HIV variation complexity was recently reported by Hahn et al by demonstrating the frequent recombination of HIV among clades (Robinson et al, J. Mol. Evol. 40:245-259 (1995)). These authors suggest that as many as 10% of HIV isolates are mosaics of recombination, suggesting that vaccines based on only one HIV clade will not protect immunized subjects from mosaic HIV isolates (Robinson et al, J. Mol. Evol. 40:245-259 (1995)).

The present invention relates to an immunogen suitable for use in an HIV vaccine. The immunogen will induce broadly cross-reactive neutralizing antibodies in humans and neutralize a wide spectrum of HIV primary isolates.

SUMMARY OF THE INVENTION

The present invention relates to an immunogen for inducing antibodies that neutralize a wide spectrum of HIV primary isolates. The invention

also relates to a method of inducing anti-HIV antibodies using such an immunogen.

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Peptide immunogen design.

Figure 2. Sequence of CBLH-1-89.6P.

DETAILED DESCRIPTION OF THE INVENTION

Targets that induce antibodies that neutralize primary isolates of HIV include the gp120 V3 loop and the CCR5 (cellular HIV co-receptor) binding site. Kwong et al (Nature 393:648-659 (1968)) have shown that the CCR5 binding site is adjacent to the base of the V3 loop and is formed by the juxtaposition of 4 anti-parallel beta-pleated sheets. The present invention provides a peptide immunogen that induces antibodies that neutralize HIV primary isolates comprising components of both the HIV gp120 CCR5 binding site and the V3 loop. The immunogen has the design set forth in Figure 1.

The peptide immunogen of the invention, designated CCR5 binding site/V3, with the CBLH-1 peptide being the prototype, comprises, from the N-terminus to the C-terminus, beta sheet regions 20, 21, 2 and 3 (see Nature 393:650 (1998)). A V3 loop sequence connects beta sheets 21 and 2 and a V3 loop

sequence is present between beta sheets 2 and 3, which site is naturally occupied by the V1-V2 loops. Accordingly, the peptide immunogen of the invention comprises 4 anti-paralleled beta sheet sequences that reflect the CCR5 binding site and 2 V3 loops. The V3 loops can vary in length (for example, from about 8 to about 16 amino acids). In a preferred embodiment, the 4 beta sheets correspond to disparate gp120 regions. In CCR5 binding site/V3, CBLH-1, they are present in a linear peptide comprising V3 loops.

A multiplicity of peptide immunogens of the present invention can be formulated as a composition suitable for administration as a vaccine. The V3 components of the peptide immunogens of the invention present in the instant composition are selected so as to be representative of higher order structural motifs present in a population, which motifs mediate V3 functions in the course of envelope mediated HIV interaction with host cells. The Los Alamos National Laboratories Human Retroviruses and AIDS Database (Human Retroviruses and AIDS, 2000, Published by the Theoretical Biology and Biophysics G T-10, Mail Stop K710, LANL, Los Alamos, NM) presently contains over 14,000 HIV V3 envelope sequences, showing the extraordinary diversity the virus has obtained since originating in man in Africa approximately 50 years ago. For example, among 432 HIV-1 V3 sequences derived from individuals infected with subtype C (designated "Clade C") in Africa currently available in the HIV

database, 176 distinct variants of a 23 amino acid stretch at the tip of the V3 loop have been found. Similarly, among 6870 B subtype (designated "Clade B") V3 sequences from the US, 1514 unique forms have been found.

A method has been developed to organize short antigenic domains by protein similarity scores using maximum-linkage clustering. This method enables the visualization of the clustering patterns as a dendrogram, and the splitting patterns in the dendrogram can be used to define clusters of related sequences (Korber et al, J. Virol. 68:6730-6744 (1994)). The method allows the use of several different amino acid similarity scoring schemes available in the literature, preferred is the amino acid substitution matrix developed by Henikoff and Henikoff (see Advances in Protein Chemistry 54:73-97 (2000) and Proteins: Structure, Function and Genetics 17:49-61 (1993)), designed to give substitutions that are well tolerated in conserved protein structural elements a high score, and a low score to those that are not. Typically excluded from consideration very rare, highly divergent peptides, and favored are peptides found in many individuals within the population. In a selected set of sequences , most of the unique forms are within one or two amino acids from a least one other of the peptides chosen. This method has been applied to clustering the large number of variants of the antigenic tip of the V3 domain within Clade B and Clade C into groups (about 25) that are likely

to be cross-reactive within the group. Based on these clustering patterns, variants (e.g., about 25-30) are selected that are representative or "central" to each group, for testing for antigenicity. The HIV Clade B and Clade C gp120 envelope V3 sequences have been analyzed, as described above, for groups of V3 sequences predicted to have structural similarities. Twenty five Clade C and 30 Clade B groups have been defined, and chosen out of each group is a common, or the most common, sequence as a representative of that group.

Shown in Tables 3 and 4 are examples of immunogens of the present invention for HIV Clades B and C, respectively. The immunogens of B can be combined to provide a composition suitable for use in the US (clade B) and Africa (Clade C).

Table 3

396.2/170.6-RIKQIINMWQKVGKAMYA-RRNIHIGLGRRF-
SLKPCVKTPLCV-RRSVRIGPGGAM-SCNTSVITQA

82.15/144.8-RIKQIINMWQKVGKAMYA-RRSIPIGPGRAF-
SLKPCVKTPLCV-VRKIPIGPGSSF-SCNTSVITQA

23.38/365.2-RIKQIINMWQKVGKAMYA-RKRIPLGLGKAF-
SLKPCVKTPLCV-RKGIHLGPGRAI-SCNTSVITQA

513.2/1448.1-RIKQIINMWQKVGKAMYA-RKGIHMGPGKAI-
SLKPCVKTPLCV-RRGIPIGPGRAF-SCNTSVITQA

69.18/146.8-RIKQIINMWQKVGKAMYA-RKSIRIGPGRAV-
SLKPCVKTPLCV-RRRISIGPGRAF-SCNTSVITQA

113.10/51.23-RIKQIINMWQKVGKAMYA-RRSIHLGMGRAL-
SLKPCVKTPLCV-RRSIHMGLGRAF-SCNTSVITQA

72.18/36.29-RIKQIINMWQKVGKAMYA-RKGINIGPGRAF-
SLKPCVKTPLCV-RKGIHIGPGRTF-SCNTSVITQA

70.18/89.14-RIKQIINMWQKVGKAMYA-IRIGHIGPGRAF-
SLKPCVKTPLCV-RRHIHIGPGRAF-SCNTSVITQA

163.7/57.20-RIKQIINMWQKVGKAMYA-RRKGIHIGPGRAI-
SLKPCVKTPLCV-TGKSIRMGLGRAW-SCNTSVITQA

11.85/34.29-RIKQIINMWQKVGKAMYA-RKSINIGPGRAF-
SLKPCVKTPLCV-RKSIQIGPGRAF-SCNTSVITQA

1.481/85.15-RIKQIINMWQKVGKAMYA-RKSIHIGPGRAF-
SLKPCVKTPLCV-RKSIHIAPGRAF-SCNTSVITQA

62.19/125.9-IKQIINMWQKVGKAMYA-RKSIHIGPGRAF-
SLKPCVKTPLCV-RRRISMGPGRVL-SCNTSVITQA

35.29/74.17-RIKQIINMWQKVGKAMYA-RKRISLGPGRVY-
SLKPCVKTPLCV-RKRMTLGPGKVF-SCNTSVITQA

46.26/122.9-RIKQIINMWQKVGKAMYA-QRIIHIGPGRPF-
SLKPCVKTPLCV-RIRIHRGYGRSF-SCNTSVITQA

162.7/3.323-RIKQIINMWQKVGKAMYA-RGSIHLHPGRKF-
SLKPCVKTPLCV-RKSINMGPGRAF-SCNTSVITQA

Table 4

1(4)-RIKQIINMWQKVGKAMYA-rksirigpGqtf-SLKPCVKTPLCV-rksVrigpGqtf-SCNTSVITQA

7(8)-RIKQIINMWQKVGKAMYA-rEsirigpGqtf-SLKPCVKTPLCV-rRsirigpGqAf-SCNTSVITQA

9(10)-RIKQIINMWQKVGKAMYA-rkGirigpGqtf-SLKPCVKTPLCV-rksirigpGqAf-SCNTSVITQA

14(15)-RIKQIINMWQKVGKAMYA-rksMrigpGqtf-SLKPCVKTPLCV-rksirigpGqtL-SCNTSVITQA

16(17)-RIKQIINMWQKVGKAMYA-rksVrigpGqtS-SLKPCVKTPLCV-rRsirigpGqtf-SCNTSVITQA

20(22)-RIKQIINMWQKVGKAMYA-rQsirigpGqAf-SLKPCVKTPLCV-rksVrigpGqAf-SCNTSVITQA

23(24)-RIKQIINMWQKVGKAMYA-rkGiHigpGqAf-SLKPCVKTPLCV-rkGiGigpGqtf-SCNTSVITQA

25(14)-RIKQIINMWQKVGKAMYA-rEsiGigpGqAf-SLKPCVKTPLCV-rksMrigpGqtf -SCNTSVITQA

While the above is offered by way of example, it will be appreciated that the same analyses can by performed for HIV Clades A, D, E, F, G, H, M, N, O, etc, to design immunogens that react with HIV primary isolates from these Clades. The length of the V3 inserts in the present immunogens can vary, for example, from about 8 to about 16 amino acids. In a similar manner, analysis can be made of amino acid heterogeneity with the 2, 3, 20 and 21 beta sheet regions of gp120 and multiple HIV (chemokine) receptor binding site sequences can be used in peptide design.

The peptide immunogens of the invention can be chemically synthesized and purified using methods which are well known to the ordinarily skilled artisan. The composition can comprise the peptides linked end to end or can comprise a mixture of individual peptides. The peptide immunogens can also be synthesized by well-known recombinant DNA techniques. Recombinant synthesis may be preferred when the peptides are covalently linked.

Nucleic acids encoding the peptides of the invention can be used as components of a DNA vaccine wherein the peptide encoding sequence(s) is/are administered as naked DNA or, for example, a minigene encoding the peptides can be present in a viral vector, such as an adenoviral vector, a modified vaccinia ankara vector, a vaccinia vector or an attenuated TB vector. Expression of the immunogenic peptides of the invention can be induced in a patient's own cells, by introduction into those cells of nucleic acids that encode the peptides, preferably using codons and promoters that optimize expression in human cells. Examples of methods of making and using DNA vaccines are disclosed in U.S. Pat. Nos. 5,580,859, 5,589,466, and 5,703,055.

The composition of the invention comprises an immunologically effective amount of the peptide immunogens of this invention, or DNA sequence(s) encoding same, in a pharmaceutically acceptable delivery system. The compositions can be used for prevention and/or treatment of immunodeficiency virus infection. The compositions of the invention

can be formulated using adjuvants, emulsifiers, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions. Optimum formulations can be readily designed by one of ordinary skill in the art and can include formulations for immediate release and/or for sustained release, and for induction of systemic immunity and/or induction of localized mucosal immunity (e.g, the formulation can be designed for intranasal administration). The present compositions can be administered by any convenient route including subcutaneous, intranasal, oral, intramuscular, or other parenteral or enteral route. The immunogens can be administered as a single dose or multiple doses. Optimum immunization schedules can be readily determined by the ordinarily skilled artisan and can vary with the patient, the composition and the effect sought. By way of example, it is noted that approximately 50μg-100μg of each hybrid peptide can be administered, for example, intramuscularly (e.g. 3x).

The invention contemplates the direct use of both the peptides of the invention and nucleic acids encoding same. For example, a minigene encoding the peptides can be used as a prime and/or boost.

In addition to the composition described above, the invention encompasses each of the immunogens disclosed as well as each of the components (V3 and CCR5), alone or in covalent or non-covalent association with other sequences. The invention

further encompasses nucleic acid sequences encoding any and all such peptides.

Certain aspects of the invention are described in greater detail in the non-limiting Example that follows.

EXAMPLE

A peptide immunogen of the invention, designated CBLH-1-89.6P) and having the sequence shown in Fig. 2 was tested for both immunogenicity with antibodies against the peptide and for neutralizing antibodies. Shown in Table 1 are the results of immunization of guinea pigs twice with CBLH-1 of SHIV89.6P in complete Freund's adjuvant (CFA)/incomplete Freund's adjuvant (IFA) versus immunization of guinea pigs twice with another immunogen, the C4-V3 gp120 immunogen (see Provisional Application No. 60/331,036).

Table 1

Animal number	Immunogen	Titer to Immunizing peptide after 2 Immunizations
322	CBLH-1 of SHIV89.6P	102,400
323	CBLH-1 of SHIV 89.6P	204,800
324	CBLH-1 of SHIV 89.6P	102,400
325	C4-V3 89.6P	25,600
326	C4-V3 89.6P	12,800
327	C4-V3 89.6P	12,800

Table 2 shows the neutralizing antibody results of the sera of the same animals against several HIV primary isolates.

Table 2

			Nab titer in MT-2 cells[1]		% p24 reduction in PBMC[2]	
Animal	Immunogen	Bleed	HIV-1MN	SHIV-89.6P	SF162	JR-FL
322	CBLH-1	Pre	0	0	0	0
		1	0	0	0	0
		2	0	0	0	0
		3	122	0	0	0
		4	75	0	0	0
323	CBLH-1	Pre	0	0	0	0
		1	42	0	0	0
		2	444	0	0	0
		3	>540	0	100	88
		4	>540	0	100	0
324	CBLH-1	Pre	0	0	0	0
		1	0	0	0	0
		2	188	0	0	0
		3	>540	0	89	0
		4	>540	24	93	0
325	C4-V3 89.6P	Pre	0	0	0	0
		1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	23	0	0
326	C4-V3 89.6P	Pre	0	0	0	0
		1	79	0	0	0
		2	131	0	0	0
		3	53	0	0	0
		4	47	0	0	0
327	C4-V3 89.6P	Pre	0	0	0	0
		1	81	0	0	0
		2		0	0	0
		3		0	0	0
		4		0	0	0

[1]*NAb titers are the reciprocal serum dilution at which 50% of cells were protected from virus-induced killing as measured by neutral red uptake.*

[2]*Samples were assayed at a 1:4 dilution in triplicate. % reduction in p24 is calculated relative to the amount of p24 produced in the presence of the corresponding prebleed sample.*

The results shown in Table 2 demonstrate that whereas C4-V3 neutralization titers were low and did not cross neutralize any HIV primary isolates, CBLH-1 of SHIV89.6P immunization of animals 323 and 342 induced antibodies that cross-neutralized HIV SF162 and animal 323 also cross-neutralized the primary isolate HIV JR-FL.

The following peptides have also been designed and may represent immunogenic truncated variants of CCR5 binding site/V3 peptide constructs:

1.481/85.15-Delta 20/21-RKSIHIGPGRAF-SLKPCVKTPLCV-RKSIHIAPGRAF-SCNTSVITQA

1.481/85.15-Delta 2-RIKQIINMWQKVGKAMYA-RKSIHIGPGRAF-RKSIHIAPGRAF-SCNTSVITQA

1.481/85.15-Delta 2/3-RIKQIINMWQKVGKAMYA-RKSIHIGPGRAF-RKSIHIAPGRAF

1.481/85.15-Delta 3-RIKQIINMWQKVGKAMYA-RKSIHIGPGRAF-SLKPCVKTPLCV-RKSIHIAPGRAF

1.481/85.15-Delta 20/21/3-RKSIHIGPGRAF-SLKPCVKTPLCV-RKSIHIAPGRAF.

* * *

All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

What is claimed is:

1. A peptide immunogen comprising, from the N-terminus to the C-terminus, beta sheet regions 20, 21, 2 and 3 of a human immunodeficiency virus (HIV) gp120 CCR5 binding site, wherein an HIV gp120 V3 loop sequence is present between said beta sheet regions 21 and 2 and between said beta sheets regions 2 and 3.

2. The peptide according to claim 1 wherein each of said V3 loop sequences comprises from about 8 to about 16 amino acids.

3. The peptide according to claim 1 wherein said beta sheet regions correspond to disparate gp120 regions.

4. A composition comprising at least two peptides according to claim 1.

5. The composition according to claim 4 wherein said at least 2 peptides are covalently linked.

6. A method of inducing an immune response in a patient to HIV comprising administering to said patient at least one peptide according to claim 1 in an amount and under conditions such that said response is induced.

7. A vaccine comprising a multiplicity of peptides according to claim 1 wherein said V3 loop sequences are selected so as to be representative of higher order structural motifs present in a population of HIV isolates.

8. The peptide according to claim 1 wherein said peptide comprises a sequence selected from the group consisting of

RIKQIINMWQKVGKAMYA-RRSIPIGPGRAF-SLKPCVKTPLCV-VRKIPIGPGSSF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKRIPLGLGKAF-SLKPCVKTPLCV-RKGIHLGPGRAI-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKGIHMGPGKAI-SLKPCVKTPLCV-RRGIPIGPGRAF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKSIRIGPGRAV-SLKPCVKTPLCV-RRRISIGPGRAF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RRSIHLGMGRAL-SLKPCVKTPLCV-RRSIHMGLGRAF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKGINIGPGRAF-SLKPCVKTPLCV-RKGIHIGPGRTF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-IRIGHIGPGRAF-SLKPCVKTPLCV-RRHIHIGPGRAF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RRKGIHIGPGRAI-SLKPCVKTPLCV-TGKSIRMGLGRAW-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKSINIGPGRAF-SLKPCVKTPLCV-RKSIQIGPGRAF-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKSIHIGPGRAF-SLKPCVKTPLCV-RKSIHIAPGRAF-SCNTSVITQA;

IKQIINMWQKVGKAMYA-RKSIHIGPGRAF-SLKPCVKTPLCV-RRRISMGPGRVL-SCNTSVITQA;

RIKQIINMWQKVGKAMYA-RKRISLGPGRVY-SLKPCVKTPLCV-
RKRMTLGPGKVF-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-QRIIHIGPGRPF-SLKPCVKTPLCV-
RIRIHRGYGRSF-SCNTSVITQA; and
RIKQIINMWQKVGKAMYA-RGSIHLHPGRKF-SLKPCVKTPLCV-
RKSINMGPGRAF-SCNTSVITQA.

9. A composition comprising at least two of said peptides according to claim 8.

10. The peptide according to claim 1 wherein said peptide comprises a sequence selected from the group consisting of
RIKQIINMWQKVGKAMYA-rksirigpGqtf-SLKPCVKTPLCV-
rksVrigpGqtf-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rEsirigpGqtf-SLKPCVKTPLCV-
rRsirigpGqAf-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rkGirigpGqtf-SLKPCVKTPLCV-
rksirigpGqAf-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rksMrigpGqtf-SLKPCVKTPLCV-
rksirigpGqtL-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rksVrigpGqtS-SLKPCVKTPLCV-
rRsirigpGqtf-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rQsirigpGqAf-SLKPCVKTPLCV-
rksVrigpGqAf-SCNTSVITQA;
RIKQIINMWQKVGKAMYA-rkGiHigpGqAf-SLKPCVKTPLCV-
rkGiGigpGqtf-SCNTSVITQA; and
RIKQIINMWQKVGKAMYA-rEsiGigpGqAf-SLKPCVKTPLCV-
rksMrigpGqtf -SCNTSVITQA.

11. A composition comprising at least two of said peptides according to claim 10.

12. A nucleic acid sequence encoding at least one peptide according to claim 1.

13. A composition comprising at least one nucleic acid sequence encoding at least two of said peptides according to claim 1.

14. A method of inducing an immune response in a patient to HIV comprising administering to said patient at least one nucleic acid sequence according to claim 12 under conditions such that said nucleic acid sequence is expressed, said at least one peptide is produced and said immune response is induced.

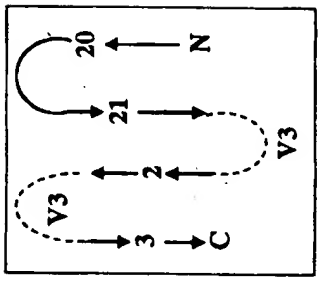

Figure 1

CBLH-1 – 89.6P =

β20	β21	V3	β2	V3	β3
RIKQIINMWQKVGKAMYA-SIGPGRAF-SLKPCVKTPLCV-SIGPGRAF-SCNTSVITQA					

Figure 2

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WO 2003/046137 A3

(54) Title: HIV ENVELOPE V3-CCR5 BINDING SITE IMMUNOGEN

(57) Abstract: The present invention relates, in general, to an immunogen and, in particular, to an immunogen for inducing antibodies that neutralize a wide spectrum of HIV primary isolates. The invention also relates to a method of inducing anti-HIV antibodies using same.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/37712

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 38/00, 38/04, 39/00, 39/21; C07K 1/00, 5/00, 7/00; C12N 15/00, 15/09, 15/63
US CL : 424/188.1, 192.1, 208.1; 530/324, 325, 326, 327, 328, 350, 826; 536/23.72
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/188.1, 192.1, 208.1; 530/324, 325, 326, 327, 328, 350, 826; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, West

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OSCHERWITZ et al. A V3 loop haptenic peptide sequence, when tandemly repeated, enhances immunogenicity by facilitating helper T-cell responses to a covalently linked carrier protein. Vaccine. 14 May 1999, Vol. 17, No. 19, pages 2392-2399, see entire document.	1-7, 9, and 11-14
Y	BORBE et al. Structural and immunological reactivity of the principal neutralizing determinant V3 of glycoprotein gp120 of HIV-1. Journal of Peptide Science. March-April 1995, Vol. 1, No. 2, pages 109-123, see entire document.	1-7, 9, and 11-14
Y	WINCHELL et al. Mucosal immune response to an HIV C4/V3 peptide following nasal or intestinal immunization of rabbits. AIDS Research and Human Retroviruses. 01 July 1997, Vol. 13, No. 10, pages 881-889, see entire document.	1-7, 9, and 11-14
Y	KELLEHER et al. Safety and immunogenicity of UBI HIV-1MN octameric V3 peptide vaccine administered by subcutaneous injection. AIDS Research and Human Retroviruses, 01 January 1997, Vol. 13, No. 1, pages 29-32, see entire document.	1-7, 9, and 11-14

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 December 2003 (05.12.2003)	15 DEC 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Jeffrey Stucker Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US02/37712

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HAYNES et al. HIV type 1 V3 region primer-induced antibody suppression is overcome by administration of C4-V3 peptides as a polyvalent immunogen. AIDS Research and Human Retroviruses. February 1995, Vol. 11, No. 2, pages 211-221, see entire document.	1-7, 9, and 11-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/37712

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 8 and 10
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 8 and 10 are found to be unsearchable under Article 17(2)(b) because these claims lack sequence identifiers and thereby cannot be searched.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/37712

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-7, 9, and 11, drawn to a peptide immunogen and method of use.

Group II, claims 12-14, drawn to a nucleic acid sequence and method of use.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The compositions of each group are chemically and structually different. The methods using each of the compositions are likewise different because each of the compositions has different characteristics and is used and administered differently.